nature portfolio

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Reporting Summary

Nature Portfolio wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Portfolio policies, see our Editorial Policies and the Editorial Policy Checklist.

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed	
☐	☒	The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒	A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☒	☐	The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☒	☐	A description of all covariates tested
☒	☐	A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒	A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☒	☐	For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☒	☐	Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	JEOL cryoARM300 microscope with 300kV accelerating voltage and equipped with a K3 direct electron detector (Gatan)
Data analysis	SerialEM 4.1.0 beta26, CryoSPARC Live, cryoSPARC-v.4, Coot 0.8.9.2, Phenix v.1.21.1-5207, DeepEMhancer, Alphafold2, Pymol v.2.0.7 and v.2.5.4, ChimeraX-1.3, eLBOW, Molprobity

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our policy

Atomic models have been deposited in the Protein Data Bank (PDB), and cryo-EM maps and corresponding half maps can be accessed through the Electron Microscopy Data Bank (EMDB). The atomic models have been deposited under accession code 9FYQ [https://doi.org/10.2210/pdb9FYQ/pdb] (SV2A-PMb5-TeNT-HC) and 9FYR https://doi.org/10.2210/pdb9FYR/pdb] (LEV-SV2A-PMb5-TeNT-HC). The corresponding maps can be found under EMD-50889 [https://www.ebi.ac.uk/pdbe/entry/emdb/EMD-50889] (global map SV2A-PMb5-TeNT-HC), EMD-50890 [https://www.ebi.ac.uk/pdbe/entry/emdb/EMD-50890] (local map 'TeNT-

Gangliosides') and EMD-50891 [https://www.ebi.ac.uk/pdbe/entry/emdb/EMD-50891] (LEV-bound SV2A-PMb5-TeNT-HC). The source data underlying Supplementary Figures 1C-E, 4A-B, 9 are provided as a Source Data file

Research involving human participants, their data, or biological material

Policy information about studies with human participants or human data. See also policy information about sex, gender (identity/presentation), and sexual orientation and race, ethnicity and racism.

Reporting on sex and gender	n/a
Reporting on race, ethnicity, or other socially relevant groupings	n/a
Population characteristics	n/a
Recruitment	n/a
Ethics oversight	n/a

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	For the cryo-EM data collection, the required number of micrographs collected was estimated based on our experience with similar proteins. From our experience we could estimate that the sample size is sufficient to enable solving the structures at high-resolution. The amount of particles in the final reconstructions is 89,713 and 162,694 for SV2A-PMb5-TeNT-HC and 158,703 for the LEV-bound structure. For the thermostability assay in the presence and absence of LEV, three technical replicates were performed, and the experiment was repeated twice with similar outcome.
Data exclusions	No biochemical data was excluded from the study
Replication	The cryo-EM data was collected once, and structures have been determined and validated. For the thermostability assay in the presence and absence of LEV, three technical replicates were performed, and the experiment was repeated twice with similar outcome.
Randomization	For cryo-EM and map processing, the data was devided into two groups (randomly splitted) and the FSC gold standard correlation was calculated. For the thermostability assay, randomization was not applicable.
Blinding	No blinding was applied (not necessary for cryo-EM and the performed assay) and all people involved in this study conducted the experiments according to their expertise.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☐	☒ Animals and other organisms
☒	☐ Clinical data
☒	☐ Dual use research of concern
☒	☐ Plants

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used	-SV2A antibody - 119 003 (Synaptic Systems, Göttingen Germany) -SV2B antibody - 119 103 (Synaptic Systems, Göttingen Germany) -SV2C antibody - 119 203 (Synaptic Systems, Göttingen Germany)
Validation	No further validation was performed

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	tsA201 cells purchased frrom Sigma/Merck (ECACC 96121229)
Authentication	No further authentication was performed after purchasing tsA201 cells from Sigma/Merck
Mycoplasma contamination	Mycoplasma contamination was not tested (The cells have been eradicated from mycoplasma at ECACC)
Commonly misidentified lines (See ICLAC register)	Commonly misinvestigated cell lines were not used in this work

Animals and other research organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research, and Sex and Gender in Research

Laboratory animals	Lama glama
Wild animals	n/a
Reporting on sex	Sex was not considered in the study design.
Field-collected samples	n/a
Ethics oversight	The project involved the generation of camelid antibodies for which llamas need to be immunized and blood samples collected. This is all done in compliance with both the European legislation (EU directive 2010/63/EC) and the Belgian Royal Decree of 29 May 2013 concerning the protection of laboratory animals with the exception that the animals are not specifically bred for such use. The llamas are housed in a center (farm), which is licensed by the Belgian competent authorities (accreditation number LA 1700601) and all staff involved is appropriately trained. The immunization and blood collection of the llamas has been approved by the authorized local Animal Ethics Review board. The approval reference number is 12-xxx-1.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Plants

Seed stocks	*Report on the source of all seed stocks or other plant material used. If applicable, state the seed stock centre and catalogue number. If plant specimens were collected from the field, describe the collection location, date and sampling procedures.*
Novel plant genotypes	*Describe the methods by which all novel plant genotypes were produced. This includes those generated by transgenic approaches, gene editing, chemical/radiation-based mutagenesis and hybridization. For transgenic lines, describe the transformation method, the number of independent lines analyzed and the generation upon which experiments were performed. For gene-edited lines, describe the editor used, the endogenous sequence targeted for editing, the targeting guide RNA sequence (if applicable) and how the editor was applied.*
Authentication	*Describe any authentication procedures for each seed stock used or novel genotype generated. Describe any experiments used to assess the effect of a mutation and, where applicable, how potential secondary effects (e.g. second site T-DNA insertions, mosiacism, off-target gene editing) were examined.*